

## First Record of Adults of *Prosimulium* and *Twinnia* (Diptera: Simuliidae) from Korea

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### ABSTRACT

Adults of *Prosimulium kiotoense* and *Twinnia japonensis* were collected and reported from Korea for the first time. Since adult black flies are notorious for morphological homogeneity particularly in female, larval and pupal characters have been mainly used to identify them. Adults of *P. kiotoense* can be identified by the following combination of characteristics: Adult, wing with radial sector (Rs) branched into R<sup>2+3</sup> and R<sup>4+5</sup>; hind leg basitarsus without calcipala; first tarsomere without pedisulcus. Female, claw without basal thumblike lobe; hypogenital valve elongate, convex, heavily sclerotized medially, posterior end touching each other, space between valves rhomboid. Male, claw with basal thumblike lobe; ventral plate keel shaped; gonostylus with 2–3 spinules. Adults and pupa of *T. japonensis* can be identified by the following combination of characteristics: Adult, antenna with 7 flagellomeres. Female, hypogenital valve broad, posterior end of valve not touching each other; cercus elongate, subquadrate; spermatheca slightly wider and long, round. Male, claw with basal thumblike lobe; gonostylus with 1 spinule; ventral plate flat. Pupa, gill of 16 filaments, arising from 3 swollen stalks; abdominal tergites without spine combs except tergites III and IV with small recurved hooks; terminal spine well developed, wavy shaped.

**Keywords:** adults, black fly, Korea, *Prosimulium*, *Twinnia*

### INTRODUCTION

Black flies (Diptera: Simuliidae) are small, nuisance insects capable of transmitting diseases to human and other animals while blood feeding from their hosts (Adler et al., 2004). Black flies have exploited nearly all flowing freshwater habitats (Adler and McCreadie, 1997; Adler et al., 2004) and 2,328 species (2,310 living and 18 fossils) are recorded from the world (Adler, 2019). There were 25 species in 4 genera, 7 subgenera of black flies known to occur in Korea including single informal species (Adler, 2019; Kim, 2020a). Since they do not cause serious medical and veterinary problems, only a handful of taxonomic studies has been conducted before 2000s (i.e., Bentinck, 1955; Takaoka, 1974; Yoon and Song, 1989, 1990) for Korean black flies. Due to morphological homogeneity of adult black flies particularly in female, larval and pupal characters have been mainly used to identify and distinguish them from each other. For this reason, adults of black flies have not been described from Korea so far, notwithstanding Bentinck (1955) provided

figures of males and females including genitalia of species occurred in Korea and Japan. Furthermore many black fly larvae remained unknown because they could not be correctly associated with the known species described from other life stages, especially adults. Molecular markers such as cytochrome c oxidase subunit I (COI) have been used successfully for species identification as well as association between unknown larva with adults of the known species in insects, including black flies (Pramual and Wongpakam, 2014). Recently, detailed description of adults of *Simulium (Boreosimulium) konoi* (Takahasi, 1950) from Korea was provided for the first time (Kim, 2020b). The genera *Prosimulium* and *Twinnia* were reported from Korea based on larvae and pupae of *Prosimulium kiotoense* Shiraki, 1935 and larvae of *Twinnia japonensis* Rubtsov, 1960 recently (Kim, 2020a). Adults of both genera have not been reported from Korea so far. In this study detailed description of adults of *P. kiotoense* and adults and pupae of *T. japonensis* accompanying with photographs were provided.

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## MATERIALS AND METHODS

The classification and morphological terminology used in the text follow those of Adler et al. (2004). Pupae of *P. kiotoense* and *T. japonensis* were hand collected from the streams and fixed in 100% EtOH in the field, then transferred to 80% EtOH. External morphology was examined under a Leica M165C dissecting microscope or Leica Z16 APO macroscope (Leica, Wetzlar, Germany). Adults were reared from pupae by placing pupae on moist filter paper in insect rearing dish in room temperature. Emerged adults were fixed in 80% EtOH. Heads and post abdomens of both male and female were removed from the body with two sets of fine forceps in 80% EtOH. Detached heads and post abdomens were placed in a vial of 10% potassium hydroxide (KOH) and boiled for 10 min at 80°C to facilitate examination of mouthparts and genitalia of male and female with phase-contrast microscopy. The preparations were examined with phase-contrast microscopy (Leica DM 2500). Z-stacked digital images were taken with a digital camera (Leica DFC 295; Leica) attached to the microscope, accompanied with Z-builder software (Leica Application Suite; Leica). Consecutive digital images in different focal planes of adults and pupae were taken with a Sony A6500 digital camera (Sony, Tokyo, Japan) attached to the Leica macroscope and the images were Z-stacked using Helicon Focus software (Helicon Soft Ltd., Ukraine).

Voucher materials including slide preparations are deposited at the Arthropod Collections of the Applied Biology Program, Division of Bio-resource Sciences, Kangwon National University, Chuncheon, Korea.

## SYSTEMATIC ACCOUNTS

Order Diptera Linnaeus, 1758

Superfamily Simulioidea Newman, 1834

Family Simuliidae Newman, 1834

Subfamily Simuliinae Newman 1834

Tribe Prosimuliini Enderlein 1921

Genus *Prosimulium* Roubaud 1906

*Prosimulium* Roubaud, 1906: 519–521 (as subgenus of *Simulium*). Type species: *Simulium hirtipes* Fries, 1824: 17–18, designated by Malloch 1914: 16.

*Hellichia* Enderlein, 1925: 203–204 (as genus). Type species: *Hellichia latifrons* Enderlein, 1925: 204 (= *Melusina macropyga* Lundstrom, 1911: 20–21), by original designation.

*Taeniopterna* Enderlein, 1925: 203 (as genus). Type species: *Melusina macropyga* Lundstrom, 1911: 20–21, by original designation.

*Mallochella* Enderlein, 1930: 84 (preoccupied) (as genus).

Type species: *Mallochella sibirica* Enderlein, 1930: 84 (= *Simulium hirtipes* Fries, 1824: 17–18), by original designation.

*Mallochianella* Vargas & Diaz Najera, 1948: 67 (substitute name for *Mallochella* Enderlein, same type species).

*Piezosimulum* Peterson, 1989: 317–318 (as genus). Type species: *Piezosimulum jeanninae* Peterson, 1989: 317–330 (= *Prosimulium neomacropyga* Peterson, 1970: 134–139), by original designation.

**Diagnosis for genus** (modified from Adler et al., 2004). Female: Claws toothless, or each with minute subbasal tooth. Hypogynial valves long (typically extended to, or beyond, middle of anal lobe), anteromedial corner produced nipple-like. Spermatheca with large unpigmented area proximally.

Male: Gonostylus in inner lateral view typically conical. Ventral plate not flattened, keel shaped; lip in lateral view typically large, prominent. Paramere narrow anteriorly, broadened into subquadrangular plate posteriorly. Median sclerite short, bifid apically.

Pupa: Gill of 9 or more filaments. Thoracic trichomes unbranched. Abdominal segment IX with a dorsal pair of long terminal spines. Cocoon with loose to densely woven sleeve, lacking definite shape (amorphous).

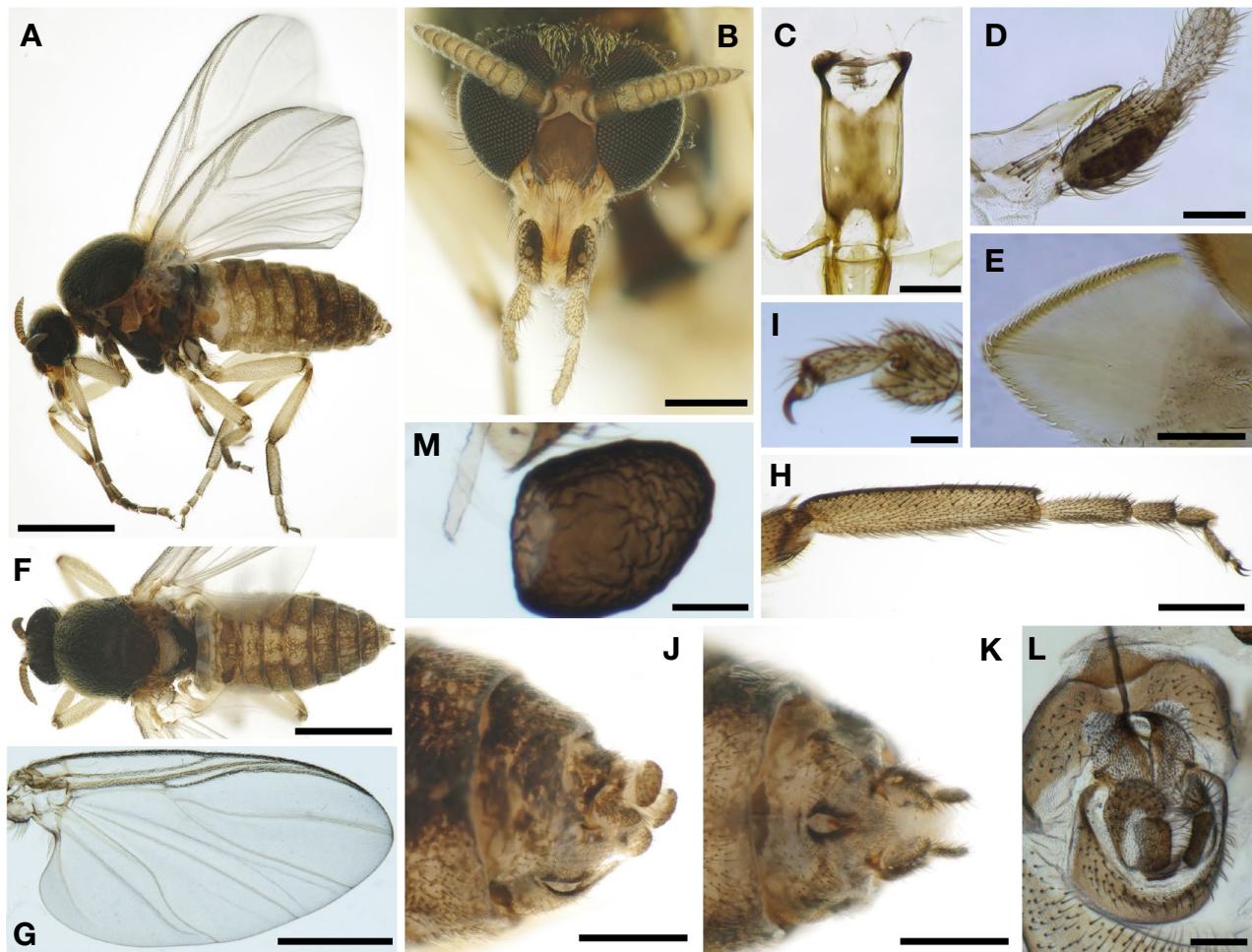
Larva: Basal two segments of antenna pale, distal segment darkly pigmented. Postgenal cleft variable, typically truncated or rounded apically. Hypostoma with median tooth trifid. Prothoracic proleg with each lateral sclerite broad, vertical portion well developed. Rectal papillae with 3 simple lobes.

### *Prosimulium kiotoense* Shiraki, 1935 (Figs. 1, 2)

*Prosimulium kiotoense* Shiraki, 1935: 6. Type locality: Kyoto, Japan.

*Prosimulium kiotoense*: Saito & Kajihara, 1975 (Japanese record); Saito et al., 1983 (Japanese record); Baba & Takaoka, 1985 (Japanese record and ecology); Baba & Takaoka, 1988 (ecology); Saito et al., 1988 (Japanese record); Baba & Takaoka, 1991a (ecology); Baba & Takaoka, 1991b (ecology); Baba & Takaoka, 1992 (ecology); Saito, 2015 (Japanese list and name); Adler, 2019 (world checklist); Kim, 2020a (larval and pupal redescription).

*Prosimulium* (*Prosimulium*) *kiotoense*: Uemoto et al., 1973 (revision); Matsuo & Uemoto, 1975 (ultrastructure); Saito & Sato, 1984 (Japanese record); Saito et al., 1985 (Japanese record); Saito et al., 1990 (Japanese record); Saito et al., 1993 (Japanese record); Saito et al., 1996b (Japanese record); Saito et al., 1996a (Japanese record); Saito & Kanayama, 2002 (Japanese record); Uemoto, 2005 (Japanese key and illustrations).



**Fig. 1.** Female of *Prosimulium kiotoense* Shiraki. A, Body profile; B, Head, frontal view; C, Cibarium, phase contrast photomicrograph; D, Part of maxillary palp and lacinia, phase contrast photomicrograph; E, Mandible, phase contrast photomicrograph; F, Body, dorsal view; G, Wing, slide mounted; H, Basitarsus and tarsomeres of hind leg, slide mounted; I, Hind leg claw, slide mounted; J, Genitalia, lateral view; K, Genitalia, ventral view; L, Genitalia, phase contrast photomicrograph; M, Spermatheca, phase contrast photomicrograph. Scale bars: A, F, G=1 mm, B, H, J, K=0.2 mm, C, D, L=100  $\mu$ m, E, I, M=50  $\mu$ m.

*Prosimulium hirtipes*, not Fries: Ogata & Sasa, 1954 (Japanese list); Bentinck, 1955 (key and illustrations); Ogata & Sasa, 1955 (Japanese key and illustration); Shogaki, 1956 (Japanese list); Ogata & Fukui, 1957 (ecology).

**Material examined.** Korea: Gangwon-do, Hongcheon-gun, Bukbang-myeon, Seongdong-ri, Gangwon Nature Environment Research Park, 37°57'48"N, 127°26'58"E, altitude 290 m, 24 Apr 2020, Kim SK (16 ultimate, 5 penultimate, 1 early instar larvae, 10 pupae, 6♂, 2♀ reared from pupa); ditto, 27 Apr 2020, Kim SK (40 ultimate, 45 penultimate, 31 early instar larvae, 100 pupae, 38♂, 22♀ reared from pupa).

**Diagnosis.** Adult: Wing with radial sector (Rs) branched into R<sup>2+3</sup> and R<sup>4+5</sup>. Hind leg basitarsus without calcipala. First tarsomere without pedisulcus.

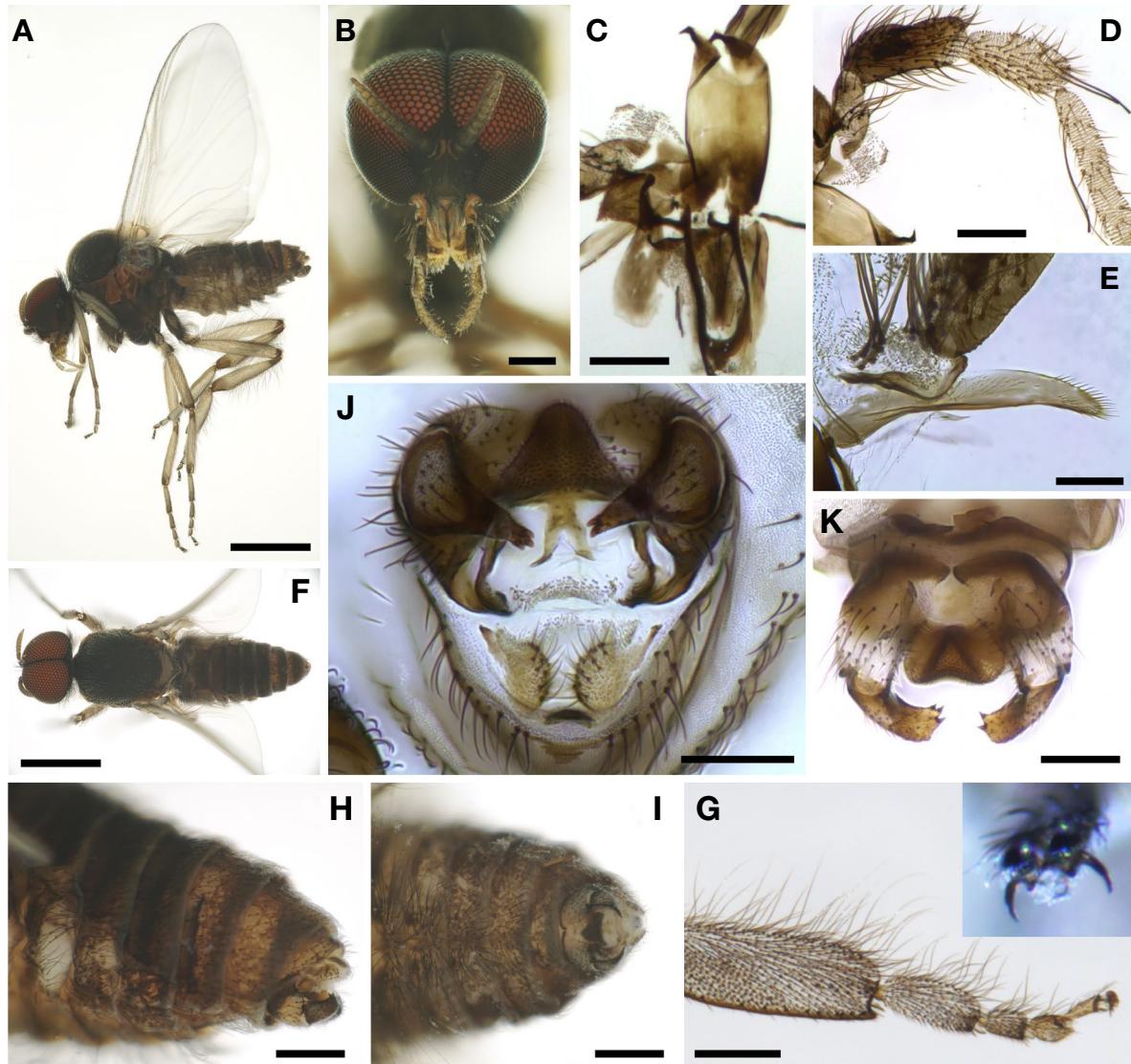
Female: Claw without basal thumblike lobe. Hypogynial valve elongate, convex, heavily sclerotized medially, posterior end touching each other, space between valves rhomboid.

Male: Claw with basal thumblike lobe. Ventral plate keel shaped. Gonostylus with 2–3 spinules.

Pupa: Gill of 16 filaments. Cocoon amorphous, entirely cover the pupa.

Larva: Antenna with proximal and medial article pale, distal article brown. Hypostoma with median tooth prominent, trifid. Postgenal cleft shallow, apex straight. Rectal papillae with 3 simple lobes.

**Description.** Female (Fig. 1). Body length (Fig. 1A, F): 3.4–3.8 mm (n=10). Head (Fig. 1B). Brown to dark brown, wider than long, as wide as or little narrower than thorax. Eye dichoptic. Antenna 11 segmented, light brown. Frons brown



**Fig. 2.** Male of *Prosimulium kiotoense* Shiraki. A, Body profile; B, Head, frontal view; C, Cibarium, phase contrast photomicrograph; D, Part of maxillary palp, phase contrast photomicrograph; E, Lacinia, phase contrast photomicrograph; F, Body, dorsal view; G, Basitarsus and tarsomeres of hind leg, slide mounted; H, Genitalia, lateral view; I, Genitalia, ventral view; J, Genitalia, phase contrast photomicrograph; K, *ditto*. Scale bars: A, F=1 mm, B, G=0.2 mm, C, D, J, K=100 µm, E=50 µm, H, I=0.5 mm.

to dark brown, densely covered with whitish golden hairs. Clypeus brown to dark brown, covered with long black setae and whitish hairs, pruinosity. Cibarium (Fig. 1C) smooth, unarmed; cornua (proximal pair of arms) long, slender. Hypopharynx broad, armed distally with numerous minute spines. Maxillary palp (Fig. 1B, D) 5 segmented, proportional ratio of 3rd to 5th segments 1 : 0.7 : 1.4; sensory vesicle 0.6 × as long as 3rd segment. Mandible (Fig. 1E) with ca. 40–50 serrations. Maxillary lacinia (Fig. 1D) with 26–30 teeth. Thorax (Fig. 1F). Wing length (Fig. 1G): 2.9–3.3 mm (n=10). Basal section of radius with dorsal hair; radial sector branched into

$R^{2+3}$  and  $R^{4+5}$ . Scutum dark brown, densely covered with whitish golden hairs. Scutellum brownish yellow, covered with long black and golden hairs. Pleural membrane (Fig. 1A) brown, bare. Katepisternum brown, bare. Legs (Fig. 1A, H). Femora brownish yellow with apically brown. Tibiae brownish yellow with basally and apically brown. Tarsi grayish brown. Hind leg basitarsus with calcipala absent. First tarsomere with pedisulcus absent. Tarsal claw (Fig. 1I) without basal thumblike lobe and subbasal tooth. Abdomen (Fig. 1A, F). Brown to dark brown, moderately covered with whitish hairs. Genitalia (Fig. 1J–M). Genital fork long and slender,

Y-shaped. Hypogynial valve long, extended to middle of anal lobe, posterior end of each valve touching each other, making space between valves pointed oblong; inner margin of each valve heavily sclerotized. Anal lobe moderately produced ventrally, broadly L-shaped, densely covered with black setae. Cercus (Fig. 1J) subquadrate, about twice as long as wide in lateral view. Spermatheca (Fig. 1M) longer than wide, surface wrinkled with no distinct polygonal patterns, large unpigmented basal area.

Male (Fig. 2). Body length (Fig. 2A, B): 3.3–3.7 (n=10). Head (Fig. 2B, C) as wide as thorax, wider than long. Eye holoptic. Upper corneal facets consisting of 19–21 vertical rows and 20–22 horizontal rows. Antenna brown, 11 segmented. Clypeus dark brown, pruinosity, moderately covered with black and whitish hairs. Cibarium (Fig. 2D) smooth, unarmed; cornua moderately long, thicker than female. Hypopharynx narrow, ca. 40 long hairs apically. Maxillary palp (Fig. 2E) 5 segmented, proportional ratio of 3rd to 5th segments 1:0.8:1.5; sensory vesicle 0.34× as long as 3rd segment. Mandible without serration. Maxillary lacinia (Fig. 2F) with ca. 35 short setae. Thorax. Wing length 2.4–2.7 (n=10). Basal section of radius with dorsal hair; radial sector branched into R<sup>2+3</sup> and R<sup>4+5</sup>. Scutum (Fig. 2B) dark brown, densely covered with whitish golden hairs. Scutellum brownish yellow, covered with long black and golden hairs. Pleural membrane brown, bare. Katepisternum brown, bare. Legs (Fig. 2A). Femora yellowish brown to brownish yellow with apically brown, with long hairs. Tibiae yellowish brown to brownish yellow with basally and apically brown, with long hairs. Tarsi yellowish brown to brownish yellow, with long hairs. Hind leg basitarsus (Fig. 2G) with calcipala absent. First tarsomere with pedisulcus absent. Tarsal claw long and slender with small basal thumblike lobe (Fig. 2G). Abdomen (Fig. 2H, I). Brown to dark brown, covered with long black and whitish hairs. Genitalia (Fig. 2J, K). Gonostylus slender, tapered to pointed apex, gently curved inwardly, with 2–3 spinules. Ventral plate with well developed median keel, triangular in posterior view. Median sclerite short, bifid apically. Paramere moderate sized, parameral spine absent. Cercus small, rounded, with 15–20 distinct hairs.

**Distribution.** Korea (Gangwon-do, Gyeonggi-do), Japan (Honshu, Kyushu).

**Stream information.** Large numbers of larvae and pupae were collected from the stream at Gangwon Nature Environment Research Park. The stream was about 10 m wide and 10–20 cm deep (Fig. 6A). Stream bed was mainly consisted of small stone and rubbles. Larvae and pupae were sporadically collected throughout the stream but majority of the pupae were collected from reed blades at single area under the bridge where the flowing water was completely covered by numerous reeds (Fig. 6B).

#### Genus *Twinnia* Stone & Jamnback

*Twinnia* Stone & Jamnback, 1955: 18–19 (as genus). Type species: *Twinnia tibblesi* Stone & Jamnback 1955: 19–21, by original designation.

#### Diagnosis for genus (modified from Adler et al., 2004).

Adult: Antenna with 7 flagellomeres.

Female: Claw toothless. Spermatheca wider than long, with large unpigmented area basally.

Male: Gonostylus with 1, rarely 2 apical spinules. Ventral plate strongly emarginated laterally near attachment of basal arms.

Pupa: Gill of 14–16 filaments, arising from 2 swollen clubs or 3 slightly swollen trunks. Abdominal tergites without spine combs; tergites III and IV with 3 pairs of recurved hooks. Cocoon transparent, gelatinous, enclosing entire pupa, retaining larval exuviae.

Larva: Cephalic apotome (frontoclypeal apotome) with median head spot extended anteriorly to level of anteriormost eyespot. Labral fans absent. Hypostoma weakly sclerotized, with broad, apically rounded teeth. Postgenal cleft absent. Anal sclerite Y shaped.

#### *Twinnia japonensis* Rubtsov, 1960 (Figs. 3–5)

*Twinnia nova japonensis* Rubtsov, 1960: Die Fliegen der palearktischen Region, 14 Simuliidae: 128. Type locality: Japan (Honshu).

*Prosimulium novum*: Bentinck, 1955, not Dyar & Shannon

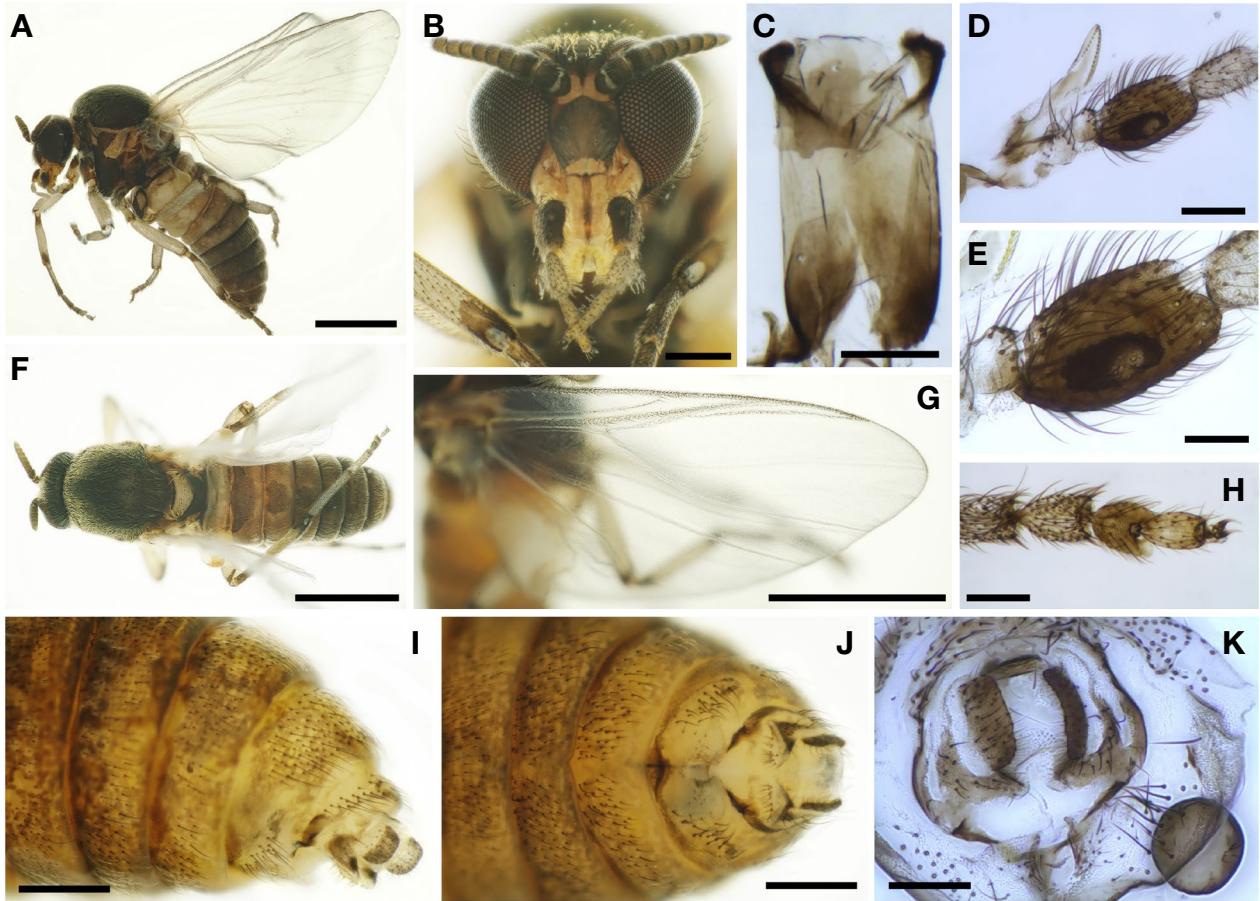
*Prosimulium (Twinnia) japonense*: Uemoto, 1980 (revision); Saito et al., 1996b (Japanese record); Uemoto, 2005 (Japanese key and illustrations).

*Twinnia japonensis*: Saito, 2015 (Japanese list and names); Adler, 2019 (world checklist); Kim, 2020a (larval description).

**Material examined.** Korea: Gangwon-do: Chuncheon-si, Namsan-myeon, Bangha-ri, 37°47'16"N, 127°32'28"E, altitude 75 m, 16 Apr 2020, Kim SK (12 ultimate, 10 penultimate, 9 early instar larvae, 1 pupa, 1♂, 2♀ reared from pupa); Chuncheon-si, Seo-myeon, Dangrim-ri, Yehyeon Hospital, 37°51'07"N, 127°37'00"E, altitude 111 m, 16 Apr 2020, Kim SK (30 ultimate, 40 penultimate, 10 early instar larvae, 6 pupae, 3♂, 3♀ reared from pupa); Hongcheon-gun, Bukbang-myeon, Seongdong-ri, Gangwon Nature Environment Research Park, 37°57'48"N, 127°26'58"E, altitude 290 m, 24 Apr 2020, Kim SK (2 ultimate, 2 early instar larvae, 1 pupa).

**Diagnosis.** Adult: Antenna with 7 flagellomeres.

Female: Hypogynial valve broad, posterior end of valve not touching each other. Cercus elongate, subquadrate. Spermatheca slightly wider and long, round.



**Fig. 3.** Female of *Twinnia japonensis* Rubtsov. A, Body profile; B, Head, frontal view; C, Cibarium, phase contrast photomicrograph; D, Part of maxillary palp and lacinia, phase contrast photomicrograph; E, Sensory vesicle of maxillary palp, phase contrast photomicrograph; F, Body, dorsal view; G, Wing, dorsal view; H, Tarsomeres of hind leg, slide mounted; I, Genitalia, lateral view; J, Genitalia, ventral view; K, Genitalia and spermatheca, phase contrast photomicrograph. Scale bars: A, F, G=1 mm, B, I, J=0.2 mm, C, D, H, K=100  $\mu$ m, E=50  $\mu$ m.

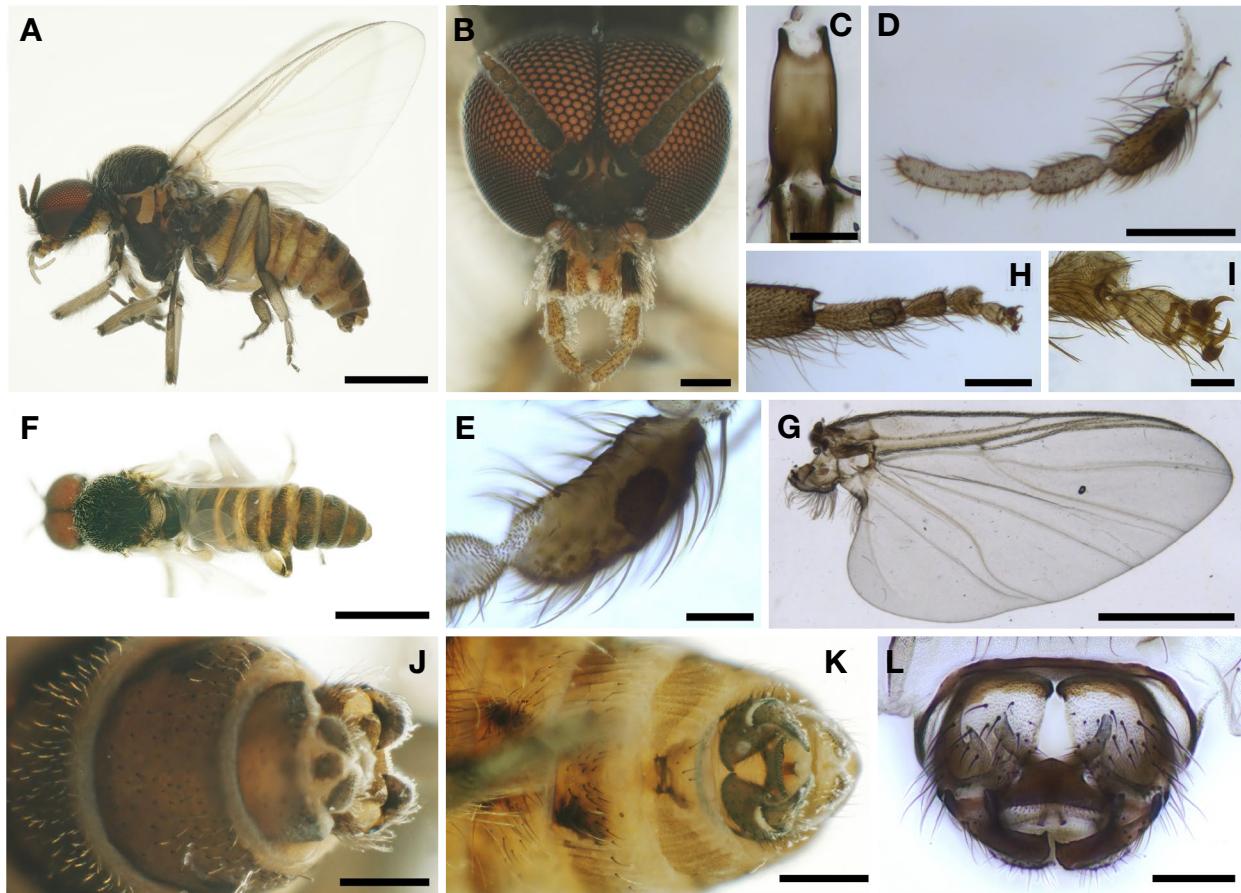
Male: Claw with basal thumblike lobe. Gonostylus with 1 spinule. Ventral plate flat.

Pupa: Gill of 16 filaments, arising from 3 swollen stalks. Abdominal tergites without spine combs; tergites III and IV with small recurved hooks. Terminal spine well developed, wavy shaped.

Larva: Labral fan absent. Postgenal cleft absent. Head with anteromedial and posterolateral head spots absent. Hypostoma with teeth broad, apically rounded, median tooth trifid. Rectal papillae with 3 simple, long, slender lobes.

**Description.** Female (Fig. 3). Body length (Fig. 3A, F): 3.4–3.7 mm ( $n=10$ ). Head (Fig. 3B). Brown to dark brown, wider than long, as wide as or little narrower than thorax. Eye dichoptic. Antenna 9 segmented, light brown. Frons brown to dark brown, densely covered with whitish golden hairs. Clypeus brown to dark brown, covered with long black setae and whitish hairs, pruinosity. Cibarium (Fig.

3C) smooth, unarmed; cornua (proximal pair of arms) long, broad. Hypopharynx broad, armed distally with numerous minute spines. Maxillary palp (Fig. 3B, D, E) 5 segmented, proportional ratio of 3rd to 5th segments 1:0.75:1.3; sensory vesicle 0.5× as long as 3rd segment. Mandible with ca. 35–40 serrations. Maxillary lacinia (Fig. 3D) with ca. 30 teeth. Thorax (Fig. 3A, F). Wing length (Fig. 3G): 2.9–3.2 mm ( $n=10$ ). Basal section of radius with dorsal hair; radial sector branched into  $R^{2+3}$  and  $R^{4+5}$ . Scutum dark brown, densely covered with whitish golden hairs. Scutellum brownish yellow, covered with long black and golden hairs. Pleural membrane (Fig. 3A) brown, bare. Katepisternum brown, bare. Legs (Fig. 3A, H). Femora brownish yellow with apically brown. Tibiae brownish yellow with basally and apically brown. Tarsi grayish brown. Hind leg basitarsus with calcipala absent. First tarsomere with pedisulcus absent. Tarsal claw (Fig. 3H) without basal thumblike lobe

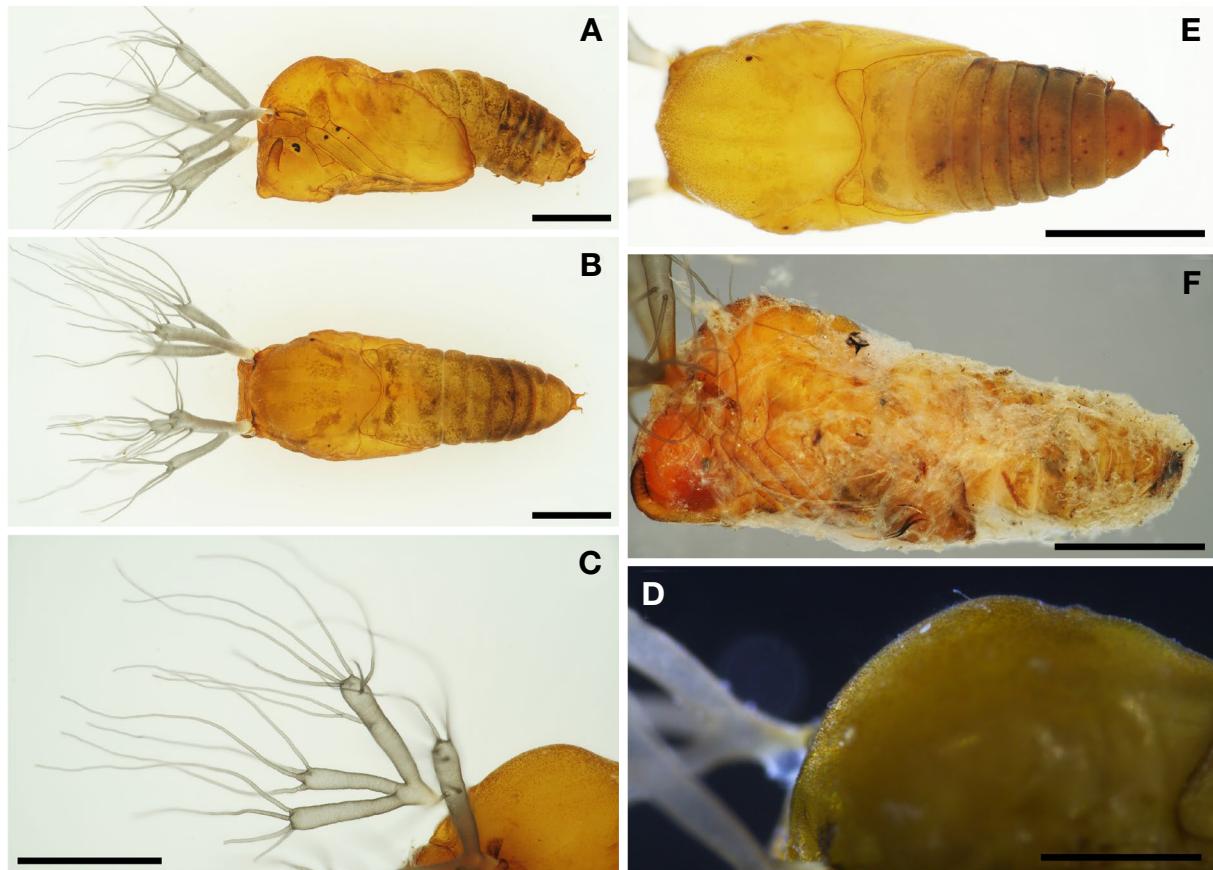


**Fig. 4.** Male of *Twinnia japonensis* Rubtsov. A, Body profile; B, Head, frontal view; C, Cibarium, phase contrast photomicrograph; D, Maxillary palp, phase contrast photomicrograph; E, Sensory vesicle of maxillary palp, phase contrast photomicrograph; F, Body, dorsal view; G, Wing, slide mounted; H, Part of basitarsus and tarsomeres of hind leg, slide mounted; I, Hind leg claw, slide mounted; J, Genitalia, posterodorsal view; K, Genitalia, ventral view; L, Genitalia, phase contrast photomicrograph. Scale bars: A, F, G=1 mm, B, D, H, J, K=0.2 mm, C, L=100  $\mu$ m, E, I=50  $\mu$ m.

and subbasal tooth. Abdomen (Fig. 3A, F). Brown to dark brown, moderately covered with whitish hairs. Genitalia (Fig. 3I–K). Genital fork long and slender, Y-shaped. Hypogenital valve broad, short, posterior end of each valve not touching each other; inner margin of each valve not heavily sclerotized. Anal lobe moderately produced ventrally, broadly L-shaped, ventrally with 10–15 brown setae. Cercus (Fig. 3I) subquadrate, about twice as long as wide in lateral view, setose. Spermatheca (Fig. 3K) round, surface wrinkled with no distinct polygonal patterns.

Male (Fig. 4). Body length (Fig. 4A, F): 3.3–3.7 (n=10). Head (Fig. 4B) as wide as thorax, wider than long. Eye holoptic. Upper corneal facets consisting of 19–21 vertical rows and 20–22 horizontal rows. Antenna brown, 11 segmented. Clypeus dark brown, pruinosity, moderately covered with black and whitish hairs. Cibarium (Fig. 4C) smooth, unarmed; cornua short. Hypopharynx narrow, ca. 35 long hairs apically.

Maxillary palp (Fig. 4D, E) 5 segmented, proportional ratio of 3rd to 5th segments 1:0.8:1.4; sensory vesicle 0.3× as long as 3rd segment. Mandible without serration. Maxillary lacinia with ca. 13–17 short setae. Thorax. Wing length 2.8–3.0 (n=10). Basal section of radius with dorsal hair; radial sector branched into  $R^{2+3}$  and  $R^{4+5}$ . Scutum (Fig. 4F) dark brown, densely covered with whitish golden hairs. Scutellum brownish yellow, covered with long black and golden hairs. Pleural membrane brown, bare. Katepisternum brown, bare. Legs (Fig. 4A). Femora yellowish brown to brownish yellow with apically brown, with long hairs. Tibiae yellowish brown to brownish yellow with basally and apically brown, with long hairs. Tarsi yellowish brown to brownish yellow, with long hairs. Hind leg basitarsus (Fig. 4H) with calcipala absent. First tarsomere with pedisulcus absent. Tarsal claw (Fig. 4I) long and slender with small basal thumblike lobe. Abdomen (Fig. 4F). Brown to dark brown, covered with long black



**Fig. 5.** Pupa of *Twinnia japonensis* Rubtsov. A, Pupa, cocoon removed, lateral view; B, Pupa, cocoon removed, dorsal view; C, Gill, lateral view; D, Thorax, dorsolateral view; E, Pupa, thorax and abdomen, dorsal view; F, Pupa with cocoon, lateral view. Scale bars: A–C, E, F=1 mm, D=0.5 mm.

and whitish hairs. Genitalia (Fig. 4J–L). Gonostylus slender, tapered to pointed apex, gently curved inwardly, with single spinule. Ventral plate flat, anvil shaped, densely covered with short seta-like hairs anteriorly. Median sclerite short, bifid apically. Paramere moderate sized, parameral spine absent. Cercus small, elongate round, with 15–20 black setae and whitish hairs.

Pupa. Body length 3.2–3.7 mm, gill length 2.2–2.5 (n=7). Pupa (Fig. 5) brown to light brown ground color. Gill (Fig. 5A–C) of 16 filaments in 3 enlarged prominent trunks; each trunk gradually thickened toward apex; uppermost trunk with 6 filaments apically and 2 filaments subapically; subapical filaments with or without short stalk; middle and lowermost trunk each with 4 filaments apically. Head with cephalic apotome light brown, densely covered with small tubercles; frontal trichome undetectable; facial trichome 1 pair, simple. Thorax (Fig. 5D) brown, densely covered with small tubercles; thoracic trichome 5 pairs, short, simple. Abdomen (Fig. 5E) densely covered with small tubercles; abdominal tergites III

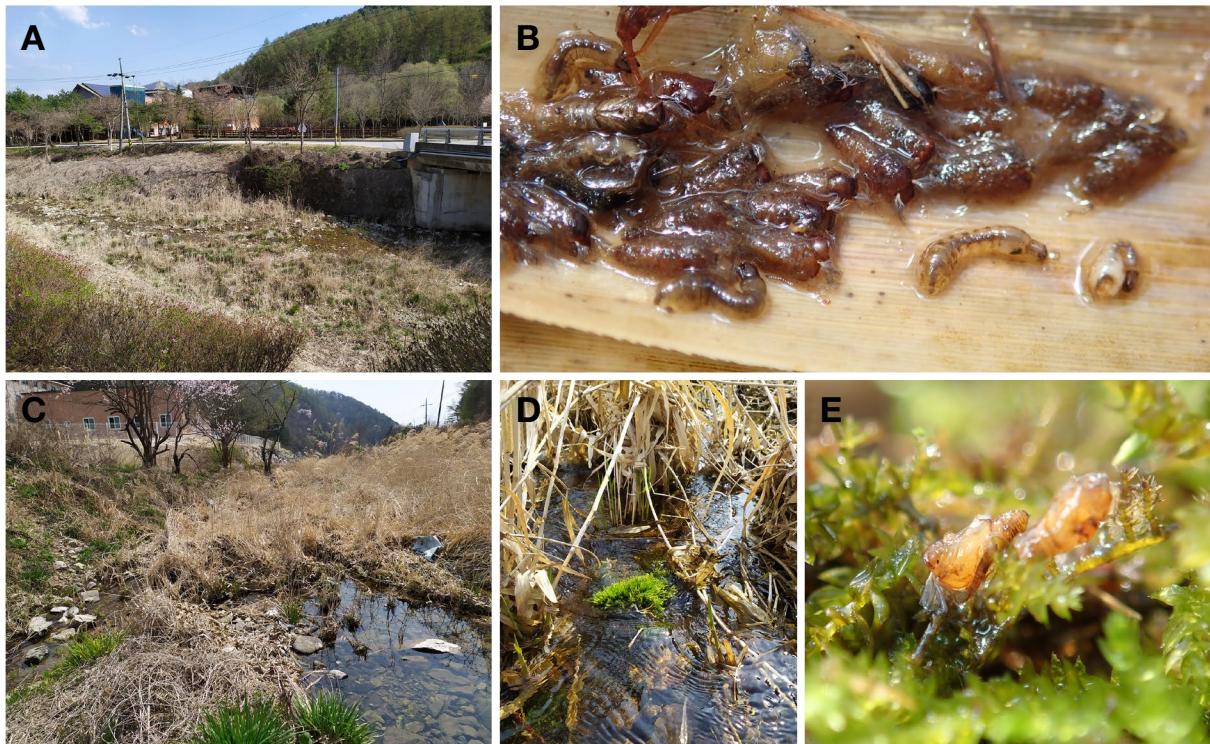
and IV apically with 3 pairs of small recurved hooks; tergites V–IX without spine comb. Terminal spine (Fig. 5E) well developed, wavy formed. Cocoon (Fig. 5F) amorphous, loosely woven, gelatinous, enclosing entire pupa, retaining larval exuviae.

**Distribution.** Korea (Gangwon-do), Japan (Honshu).

**Stream information.** Stream where the majority of larvae and pupae of *T. japonensis* were collected was small sized stream (5 m wide and 10–30 cm deep) with well developed reed (Fig. 6C). Water temperature was 11.3–14.1°C. Stream bed was consisted of small stones and ripples. Pupae were not firmly attached to the substrates, such as small stones and reed blades. Majority of larvae and pupae were collected from moss on the small stone and reed blades (Fig. 6D, E).

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**Fig. 6.** Field pictures of streams where pupae of *Prosimulium kiotoense* Shiraki and *Twinnia japonensis* Rubtsov were collected. A, Stream at Gangwon Nature Environment Research Park, Hongcheon-gun, Gangwon-do, Korea; B, Pupae of *P. kiotoense* attached to reed blade; C, Stream at Yehyeon Hospital, Chuncheon-si, Gangwon-do, Korea; D, E, Pupae of *T. japonensis* attached to the moss in the stream.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

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